**NCD FINAL ENGINEERING REPORT (03/23/21)**

STANDARD REVIEW ENGINEERING REPORT [redacted]

TERA: R21-0001 ENGINEER: Avcin/MLS (JAS Review)

SUBMITTER: [redacted]

MICROORGANISMS:

Recipients/hosts (p. 3):

[redacted]

Donor:

The donor strain is [redacted] strain [redacted], which was modified to improve nitrogen fixation through the [redacted] involved in the [redacted] (p. 2). The genetic elements that were incorporated into the recipient strain are listed in Table 3 of the submission (p. 5). A summary of that table follows:

i. [redacted]

x. [redacted]

xix. [redacted]

GEM: The submission includes [redacted] engineered strains, which are listed in Table 2 of the submission. (p. 4).

i. [redacted]

PV (CFU/yr): $1.5 \times 10^{14}$ CFU/yr (per strain)

Basis: Submitter states approximately 15 L of formulated material with a concentration of $1 \times 10^{10}$ colony-forming units (CFU)/g will be produced each year for each strain (p. 28). This is approximately $1.5 \times 10^{14}$ CFU/year per strain (p. 28).

PV = (15 L/yr) x (1000 mL/L) x ($1 \times 10^{10}$ CFU/g) x (1 g/mL)

$= 1.5 \times 10^{14}$ CFU/yr (per strain)

USE:

The objectives of the field trials are to evaluate the ability of the subject microorganisms to affect nitrogen fixation and enhance nitrogen acquisition in corn plants. (p. 27)

SUMMARY:

The specific insertions / deletions (and corresponding effects) are discussed in detail on pages 4-27 of the submission.

The subject organisms will be produced and formulated into a seed coating in the [redacted] facility. The seed coating formulation may be either liquid or solid. The seed coating formulation is then sent to the [redacted] facility where the subject organisms will be applied to corn seeds (p. 28 and technical contact). The seed coating formulation (in liquid form) may also be sent to the field test sites. Either the seeds containing the subject organisms or the seed treatment formulation will be sent to the [redacted] test sites in [redacted] and [redacted] (p. 29). At these test sites, the coated seeds will be planted or the seed treatment formulation will be applied to the seeds in-furrow (injected into the ground during seed planting) (see technical contact report), which will occur in spring 2021 (p. 29). NCD assumes 100% release to land.

NOTES AND KEY ASSUMPTIONS

The 1997 Generic Scenario for Biotechnology Premanufacture Notices was referenced in this IRER. In addition NCD referenced the May 12, 2000 technical policy memorandum on "Efficiency of Autoclaves for Laboratory-Scale Equipment" which assumes that for cases involving steam sterilization of laboratory scale equipment (<10 liters) in an autoclave, NCD will regard the potential for release of live microorganisms as negligible.

No past cases have used the same recipient strains.

[redacted]

seeds that are planted). Different submitter, different strain TERA [redacted] involved coating of the TERA onto seeds which were subsequently planted. Different submitter, different strain MCAN J18-0044 involved handing of the GEMs in solid form.

- Releases and exposures during laboratory operations (production and seed coating):
  - [redacted], [redacted], and [redacted] all assessed releases from equipment cleaning as negligible.
  - [redacted] assessed dust release to WATER/AIR/LANDFILL/INCINERATION from handling solid subject organisms.
  - [redacted], [redacted], and [redacted] all assessed inhalation and dermal exposure from handling liquid subject organisms in a laboratory setting.
  - [redacted] assessed inhalation exposure to solid subject organisms with the Small Volume Handling model.
  - This IRER assesses fermentation off-gas, equipment cleaning release, and dust release according to the June 2020 EPA/OPPT Solids Transfer Dust Transfer Model (with releases for uncaptured dust, captured and controlled dust, and captured and uncontrolled dust). This IRER assesses inhalation and dermal exposures to liquid TERA, and inhalation and dermal exposures to solid TERA.
- Releases and exposures during seed planting or in-furrow application:
  - [redacted] and [redacted] assessed releases from equipment cleaning.
  - [redacted] and [redacted] assessed a 100% release scenario due to land application. [redacted] assessed spray application to AIR or LAND (not applicable to these subject organisms) and [redacted] assessed all LAND application from seed planting (consistent with these subject organisms).
  - The use of [redacted] is unrelated to land application.
  - [redacted] assessed inhalation exposure to spray application (not applicable to these subject organisms) and dermal exposure from handling liquid subject organisms.

- o [redacted] did not assess inhalation or dermal exposure to coated seeds because the subject organisms were entrained in the coating.
- o This IRER assesses equipment cleaning release to LAND/INCINERATION, seed planting / in-furrow application release to LAND, dermal exposures to liquid TERA (in the event that the test sites receive liquid coating formulation), and does not assess inhalation and dermal exposures to solid subject organisms because they are entrained in the seed coating.

EPA assesses a 100% release scenario. The TERA will be applied to seeds, which will be planted at field test sites. Release and exposure estimates are provided on a per strain basis. Technical contact was called – see contact report.

[redacted]

The submission and technical contact indicate that the recipient strain, [redacted] strain, is [redacted]

INITIAL REVIEW ENGINEERING REPORT [redacted]

TERA: R21-0001

Manufacturing: Laboratory Propagation

Number of Sites/Locations: [redacted]

[redacted]

Days/yr: 4

Basis: The submission states that production of the subject organisms takes place over 3 to 4 days (pg. 28 and contact report).

**PROCESS DESCRIPTION:**

The subject organisms are produced as follows (p. 28):

[redacted]

**ENVIRONMENTAL RELEASE SUMMARY**

Submission did not estimate releases during laboratory propagation. NCD assesses releases per standard methodology from the Biotech GS.

The submission indicates that all waste at [redacted] sites are devitalized prior to final disposal (p. 31), which the technical contact estimates is nearly 100% effective (see contact report). Specifically, [redacted] and then released into the submitter's waste-water ponds where liquid evaporates or seeps into the ground (p. 31 and contact report). Solid waste is collected in autoclavable plastic biohazard bags, double bagged, and autoclaved for a minimum of 20 minutes at 120 degrees C prior to disposal. The autoclaved material is collected and sent to a landfill (p. 31).

The following table summarizes the total releases estimated for this operation. [redacted] NCD assesses release from emissions of [redacted] containing subject organisms during transfers of [redacted]. Technical contact indicates that handling operations occur in biosafety cabinets that are ventilated to air filters (see contact report). Per submission, all wastes are deactivated prior to disposal, with solid wastes such as filters being autoclaved to deactivate particles and then sent to landfill. The release from solids handling is assessed below per the [redacted] Model:

- Media of release for uncaptured particles = air, water, incineration, or landfill (per Dust Model)
- Media of release for captured and controlled (filtered) particles = autoclaved then sent to landfill (p. 31)
- Media of release for captured and uncontrolled particles = stack air (per Dust Model)

Additional releases are from fermentation off-gas and fermentor equipment cleaning, because the fermenter vessel is [redacted], which exceeds the GS assumption of negligible releases for laboratory-scale equipment (<10L).

**Summary of the Total Release Estimates to Environment:**

| Release | Media | Amount Released (per strain) |
|---|---|---|
| Dust – uncaptured portion | Air, water, landfill, incineration | [redacted] CFU/yr (1 site, 4 days/site-yr) or [redacted] CFU/site-day |
| Dust – captured and controlled portion | Landfill | [redacted] CFU/yr (1 site, 4 days/site-yr) or [redacted] CFU/site-day |
| Dust – captured and uncontrolled portion | Stack Air | [redacted] CFU/yr (1 site, 4 days/site-yr) or [redacted] CFU/site-day |
| Fermenter off-gas | Stack Air | [redacted] CFU/yr (1 site, 4 days/site-yr) or [redacted] CFU/site-day |
| Equipment cleaning | Incineration | [redacted] CFU/yr (1 site, 4 days/site-yr) or [redacted] CFU/site-day |
| **Total** | **All** | [redacted] **CFU/yr** |

FUGITIVE AIR/WATER/LANDFILL/INCINERATION:

1) From: Handling solid seed coating – uncaptured particles

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)(1 site, 4 days/site-yr)

Basis: Submission does not estimate releases solid handling operations. NCD uses the total PV and the 0.5% residual model (per EPA/OPPT Solids Transfer Dust Transfer Model) to calculate these releases. Technical contact indicates that operations occur in a biosafety cabinet. The Dust Model does not have capture efficiency information for biosafety cabinets, so NCD uses the default capture efficiency for a laboratory fume hood of 95%.

- [redacted] CFU/yr (PV per strain, see calculations above)
- 4 day/yr (p. 28)

- 0.5% loss to water, air, incineration, or landfill (per Dust Model)
- Biosafety cabinet capture efficiency of 95% (per Dust Model for laboratory fume hoods)

Calculations:

Annual total:

= (PV) x (0.5% dust release) x (1- capture efficiency)

= [redacted] CFU/yr) x (0.005) x (1-0.95)

= [redacted] CFU/year (per strain)

Per day:

= [redacted] CFU/year)/(4 days/yr)/(1 site)

= [redacted] CFU/site-day (per strain)

OTHER LANDFILL:

1) From: Handling solid seed coating – captured and controlled particles

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)(1 site, 4 days/site-yr)

Basis: Submission does not estimate releases solid handling operations. NCD uses the total PV and the 0.5% residual model (Dust Model) to calculate these releases. Technical contact indicates that operations occur in a biosafety cabinet. The Dust Model does not have capture efficiency information for biosafety cabinets, so NCD uses the default capture efficiency for a laboratory fume hood of 95%. Air emissions from the biosafety cabinets are ventilated through HEPA filters. NCD uses the default control efficiency for HEPA filters of 99.97% from the

Dust Model. Submission and technical contact state that any solid wastes, such as HEPA filters, are autoclaved and double bagged for disposal (pg. 31 and contact report).

- [redacted] CFU/yr (PV per strain, see calculations above)
- 4 day/yr (p. 28)
- 0.5% loss to water, air, incineration, or landfill (per Dust Model)
- Biosafety cabinet capture efficiency of 95% (per Dust Model for laboratory fume hoods)
- HEPA filter control efficiency of 99.97% (per Dust Model)
- Autoclaving sterilization efficiency 99.9999% (per GS)

Calculations:

Annual total:

= (PV) x (0.5% dust release) x (capture efficiency) x (control efficiency) x (1 – sterilization efficiency)

= [redacted] CFU/yr) x (0.005) x (0.95) x (0.9997) x (1-0.999999)

= [redacted] CFU/year (per strain)

Per day:

= [redacted] CFU/year)/(4 days/yr)/(1 site)

= [redacted] CFU/site-day (per strain)

2) From: Filters used for fermenter off-gas emissions

Amount: Negligible

Basis: Air releases could occur from exhaust gases from the [redacted] fermenter vessel. It is expected that the exhaust air from the vessels would contain liquid entrained from the fermentation broth. The entrained liquid would

contain the subject cells. The entrained liquids would be captured in the facility's air filters. Air emissions are ventilated through HEPA filters (see contact report). Submission and technical contact state that any solid wastes, such as HEPA filters, are autoclaved and double bagged for disposal (pg. 31 and contact report). Given the autoclaving sterilization efficiency (99.9999% per GS), this release is negligible (is equal to <1 CFU/yr) compared to the captured and controlled dust release.

3) Other solid wastes

Amount: negligible

Basis: No other sources of release to this medium have been identified other than potential releases from residue in equipment and PPE. Submission and technical contact state that any solid wastes, including PPE, are autoclaved and double bagged for disposal (pg. 31 and contact report). Per GS, autoclaving and the small volume of waste expected results in negligible releases.

STACK AIR:

1) From: Handling solid seed coating – captured and uncontrolled particles

Amount:

 CFU/yr (per strain)
CFU/site-day (per strain)(1 site, 4 days/site-yr)

Basis: Submission does not estimate releases solid handling operations. NCD uses the total PV and the 0.5% residual model (Dust Model) to calculate these releases. Technical contact indicates that operations occur in a biosafety cabinet. The Dust Model does not have capture efficiency information for biosafety cabinets, so NCD uses the default capture efficiency for a laboratory fume hood of 95%. Air emissions from

the biosafety cabinets are ventilated through HEPA filters. NCD uses the default control efficiency for HEPA filters of 99.97% from the Dust Model.

- [redacted] CFU/yr (PV per strain, see calculations above)
- 4 day/yr (p. 28)
- 0.5% loss to water, air, incineration, or landfill (per Dust Model)
- Biosaftey cabinet capture efficiency of 95% (per Dust Model for laboratory fume hoods)
- HEPA filter control efficiency of 99.97% (per Dust Model)

Calculations:

Annual total:

= (PV) x (0.5% dust release) x (capture efficiency) x (1-control efficiency)

= [redacted] CFU/yr) x (0.005) x (0.95) x (1-0.9997)

= [redacted] CFU/year (per strain)

Per day:

= [redacted] CFU/year)/(4 days/yr)/(1 site)

= [redacted] CFU/site-day (per strain)

2) From: Fermenter off-gas

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)(1 site, 4 days/site-yr)

Basis: Air releases could occur from exhaust gases from the [redacted] fermenter vessel. It is expected that the exhaust air from the vessels would contain liquid entrained from the

fermentation broth. The entrained liquid would contain the subject cells. The entrained liquids would be captured in the facility's air filters. Air emissions are ventilated through HEPA filters (see contact report). NCD uses the default control efficiency for HEPA filters of 99.97% from the Dust Model.

- 2 fermentations/yr (p. 28)
- 4 days/yr (p. 28)
- Batch volume = [redacted]batch (pg. 28)
- [redacted] CFU/mL (batch concentration, p. 28)
- Aerosolization factor of 1 X $10^{-9}$ (GS default)
- HEPA filter control efficiency of 99.97% (per Dust Model)

Calculations:

Annual total:

= (Vol.) x (Conc.) x (aerosolization factor) x (bt/yr) x (1-control efficiency)

= [redacted]batch) x (1000 mL/L) x [redacted] CFU/mL) x (1 X $10^{-9}$) x (2 batches/yr) x (1 – 0.9997)

= 1.8 x $10^{2}$ CFU/year (per strain)

Per day:

= (1.8 x $10^{2}$ CFU/year)/(4 days/yr)/(1 site)

= 4.5 x $10^{1}$ CFU/site-day (per strain)

OTHER INCINERATION:

1) From: Equipment cleaning wastewater

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)(1 site, 4 days/site-yr)

Basis: Submission does not explicitly address equipment cleaning. NCD's standard assumption that releases are negligible for cleaning of laboratory-scale equipment (<10 liters) is not applicable because the fermentation vessel is [redacted]. NCD assesses this release per the GS. Per submission, all liquid waste containing the TERA is treated with Lysol or Wescodyne prior to disposal, which the technical contact estimates is nearly 100% effective (see contact report). NCD assumes this is at least a 6-log reduction.

**Amendment received on 03/22/21 indicates that all liquid waste is picked up by [redacted] and incinerated.**

- 2 fermentations/yr (p. 28)
- 4 days/yr (p. 28)
- Batch volume = [redacted]batch (pg. 28)
- [redacted] CFU/mL (batch concentration, p. 28)
- 1% residual for fermentation vessel (GS)
- Per submission details described above, an inactivation efficiency of at least 99.9999% (6-log).

Calculations:

Annual total:

= (Vol.) x (Conc.) x (1% residual) x (bt/yr) x (1-control efficiency)

= [redacted]batch) x (1000 mL/L) x ([redacted]) x (0.01) x (2 batches/yr) x (1 – 0.999999)

= 6.0 x $10^6$ CFU/year (per strain)

Per day:

= (6.0 x $10^6$ CFU/year)/(4 days/yr)/(1 site)

= 1.5 x $10^6$ CFU/site-day (per strain)

**OCCUPATIONAL EXPOSURE**

Submission does not provide worker exposure estimates for seed coating formulation and application. These operations occur in a laboratory setting with the use of biosafety cabinets (see contact report). NCD assumes that some inhalation and dermal exposure in the laboratory setting as a worst case for potential sampling and transfer activities within the biosafety cabinets. Because the subject organisms may be in either solid or liquid form, NCD assesses inhalation and dermal exposures to both forms.

Number of Total Workers: 3

Basis: The submission estimates that 2 to 3 employees will be involved in the production of the TERA (NCD assumes 3 employees) (p. 28).

Days/yr: 4

Basis: The submission estimates that production will occur over 3 to 4 days (NCD assumes 4 days) for 2 to 3 hours per day (p. 28).

PPE: The submission indicates that workers wear PPE (p. 28). The technical contact clarifies that PPE includes lab coats, gloves, goggles and that workers work within biosafety cabinets (see p. 28 and contact report).

**Summary of the Occupational Exposure Estimates:**

| Worker Activity | Exposure |
|---|---|
| **Inhalation** | |
| Laboratory activities | 7-22 CFU/day (4 days/site-yr)<br>Up to 3 workers |
| Handling solid coating | 1.8 X $10^6$ to 6.0 X $10^6$ CFU/day (4 days/site-yr)<br>Up to 3 workers |
| **Dermal** | |
| Handling liquids – bench scale | [redacted]/day (4 days/site-yr)<br>Up to 3 workers |
| Handling solids | [redacted]CFU/day (4 days/site-yr)<br>Up to 3 workers |

INHALATION:

1) From: Laboratory activities - culture transfer, preparing the inoculum, and monitoring the growth.

   3 workers, 4 days/year
   7-22 CFU/day

Basis:

Although air releases from the laboratory are expected to be negligible and inhalation exposures may be mitigated by use of standard aseptic techniques, NCD's biotech generic scenario recommends assuming some inhalation exposures in a laboratory setting as a worst case. The recommended method for estimating potential inhalation exposures is to take the most applicable area monitoring data collected by NIOSH in a fermentation facility and multiply it by an estimate of the exposure duration. The NIOSH study listed the CFU concentration in a laboratory setting to be in the range from 32 to 103 CFU/$m^3$. Consistent with other recent GEM evaluations, the assumptions (and corresponding data) for exposure from 10 minutes of pipetting were used as analogous data to represent potential exposures to the microorganisms of this TERA during laboratory operations.

- $[CFU]_{WA}$ = 32 - 103 CFU/$m^3$ (GS estimate for laboratory setting)

- I (inhalation rate)= 1.25 $m^3$/hr
- H = hours per day = 10 min = 0.167 hrs (NCD assumption). This is area monitoring data in contrast to personal breathing zone monitoring. In using area monitoring data, an assumption needs to be made about the duration the worker is proximal to the location of the bioaerosol. NCD typically assumes a short duration of time for the worker to be exposed to bioaerosols during this part of the process.
- 4 days/yr (p. 28)
- 3 workers/site (p. 28)

Calculation:

$E_I = (I)x(h)x([CFU]_{WA})$ (per GS)

$= (1.25\ m^3/hr)x(0.167\ hr/day)x(32 - 103\ CFU/m^3)$

= 7-22 CFU/day

2) From: Handling solid coating

Amount of Exposure:

3 workers, 4 days/yr
$1.8 \times 10^6$ to $6.0 \times 10^6$ CFU/day

Basis:

NCD assumes that potential inhalation exposure may occur during handling of the solid coating. NCD uses the EPA Small Volume Handling Model to estimate exposures.

- 0.0477 to 0.161 mg exposure/kg solid handled (EPA Small Volume Handling Model, less than 54 kg of solid containing the PMN handled per site-day)
  - [redacted] CFU/yr (PV per strain, see calculations above)
  - [redacted] CFU/g (concentration of dried powder may be up to five times more

concentrated than the liquid material; p. 28)

- [redacted] produced per day [calc: [redacted]

- 4 day/yr (p. 28)
- 3 workers (p. 28)

Calculation:

= (0.0477 to 0.161 mg exposure/kg solid handled) x [redacted] handled/day) x (kg/1000g) x ([redacted] CFU/g) x (g/1000 mg)

= 1.8 X $10^6$ to 6.0 X $10^6$ CFU/day

DERMAL:

1) From: Bench scale handling of liquids

Amount of Exposure:
3 workers, 4 days/yr
[redacted] CFU/day

Basis:

Biotech generic scenario. The potential dermal dose rate is the product of NCD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream. For bench scale handling of liquids, the NCD standard dermal factor is <1.1 ml/day. This factor can be used with the concentration of the TERA:

- [redacted] CFU/ml (p. 28)
- C = liquid transfer - 1 hand, the NCD standard dermal factor is (535 $cm^2$/day)(0.7 to 2.1 mg/$cm^2$)(1 g/1000 mg)(1 mL/g) = 0.4 to 1.1 mL/day (NCD assumes 1 hand transfer for laboratory)
- 4 days/yr (p. 28)
- 3 workers/site (p. 28)

Calculation:

$E_D = ([CFU]_P) x C$ (per GS)

= [redacted] CFU/ml) x (<1.1 ml/day)

= [redacted] CFU/day

2) From: Handling of solids

Amount of Exposure:
3 workers, 4 days/yr
[redacted] CFU/day

Basis:
Per the biotech GS, the potential dermal dose rate is the product of NCD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream.

- [redacted] CFU/ml in solid (p. 28)
- C = solid (NCD assumes 2 hand transfer for transfers), the NCD standard dermal factor is (3,100 mg/day)(1 g/1000 mg)(1 mL/g) = up to 3.1 mL/day
- 4 days/yr (p. 28)
- 3 workers/site (p. 28)

Calculation:
$E_D = ([CFU]_P) \times C$ (per GS)

= [redacted] CFU/mL) x (3.1 mL/day)

= [redacted] CFU/day

INITIAL REVIEW ENGINEERING REPORT [redacted]

TERA: R21-0001

Processing: Seed Coating

Number of Sites/Locations: [redacted]

[redacted]

Days/yr: 2

Basis: The submission states that seed coating occurs over [redacted] days per year (pg. 28 and contact report).

**PROCESS DESCRIPTION:**

Per submission, the subject organisms will be applied to seeds either by seed treatment or in-furrow (p. 28). The technical contact clarified that the submitter intends the subject organisms to be coated onto seeds at the [redacted] facility, but there is a potential for the formulation to be applied during seed planting at the field test sites (p. 28 and contact report).

If seed coating occurs at the [redacted] site, the formulation may be received from the [redacted] site as either a solid or liquid (p. 28). The test material will have a concentration of about [redacted] CFU/mL in liquid and [redacted] CFU/mL in solid (p. 28). [redacted]

**ENVIRONMENTAL RELEASE SUMMARY**

Submission did not estimate releases during this process. The technical contact does not expect release from the coating process since coating is performed by mixing in a vessel (see contact report). NCD assesses releases per standard methodology from the Biotech GS and past case [redacted].

The submission indicates that all waste at [redacted] sites are devitalized prior to final disposal (p. 31), which the technical contact estimates is nearly 100% effective (see contact report). Specifically, liquid waste containing TERA are treated with bleach to deactivate and then released into the submitter's waste-water ponds where liquid evaporates or seeps into the ground (p. 31 and contact report). Solid waste is collected in autoclavable plastic biohazard bags, double bagged, and autoclaved for a minimum of 20 minutes at 120 degrees C prior to disposal. The autoclaved material is collected and sent to a landfill (p. 31).

The following table summarizes the total releases estimated for this operation. Because the seed coating formulation may be in solid powder format, NCD assesses release from emissions of solid particles containing subject organisms during transfers of the solid coating formulation. Technical contact indicates that handling operations occur in biosafety cabinets that are ventilated to air filters (see contact report). Per submission, all wastes are deactivated prior to disposal, with solid wastes such as filters being autoclaved to deactivate particles and then sent to landfill. The release from solids handling is assessed below per the Dust Model:

- Media of release for uncap tured particles = air, water, incineration, or landfill (per Dust Model)
- Media of release for captured and controlled (filtered) particles = autoclaved then sent to landfill (p. 31)
- Media of release for captured and uncontrolled particles = stack air (per Dust Model)

NCD assumes that the plastic food grade containers used for seed coating are small-scale (<10 L).

**Summary of the Total Release Estimates to Environment:**

| Release | Media | Amount Released (per strain) |
|---|---|---|
| Dust – uncaptured portion | Air, water, landfill, incineration | [redacted] CFU/yr (1 site, 2 days/site-yr) or [redacted] CFU/site-day |
| Dust – captured and controlled portion | Landfill | [redacted] CFU/yr (1 site, 2 days/site-yr) or [redacted] CFU/site-day |
| Dust – captured and uncontrolled portion | Stack Air | [redacted] CFU/yr (1 site, 2 days/site-yr) or [redacted] CFU/site-day |

| Release | Media | Amount Released (per strain) |
|---|---|---|
| **Total** | **All** | [redacted] **CFU/yr** |

FUGITIVE AIR/WATER/LANDFILL/INCINERATION:

1) From: Handling solid seed coating - uncaptured particles

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)(1 site, 2 days/site-yr)

Basis: Submission does not estimate releases solid handling operations. NCD uses the total PV and the 0.5% residual model (per EPA/OPPT Solids Transfer Dust Transfer Model) to calculate these releases. Technical contact indicates that operations occur in a biosafety cabinet. The Dust Model does not have capture efficiency information for biosafety cabinets, so NCD uses the default capture efficiency for a laboratory fume hood of 95%.

- [redacted] CFU/yr (PV per strain, see calculations above)
- 2 day/yr (p. 28)
- 0.5% loss to water, air, incineration, or landfill (per Dust Model)
- Biosafety cabinet capture efficiency of 95% (per Dust Model for laboratory fume hoods)

Calculations:

Annual total:

= (PV) x (0.5% dust release) x (1- capture efficiency)

= [redacted] CFU/yr) x (0.005) x (1-0.95)

= [redacted] CFU/year (per strain)

Per day:

= ([redacted] CFU/year)/(2 days/yr)/(1 site)

= [redacted] CFU/site-day (per strain)

OTHER LANDFILL:

1) From: Handling solid seed coating – captured and controlled particles

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)(1 site, 2 days/site-yr)

Basis: Submission does not estimate releases solid handling operations. NCD uses the total PV and the 0.5% residual model (Dust Model) to calculate these releases. Technical contact indicates that operations occur in a biosafety cabinet. The Dust Model does not have capture efficiency information for biosafety cabinets, so NCD uses the default capture efficiency for a laboratory fume hood of 95%. Air emissions from the biosafety cabinets are ventilated through HEPA filters. NCD uses the default control efficiency for HEPA filters of 99.97% from the Dust Model. Submission and technical contact state that any solid wastes, such as HEPA filters, are autoclaved and double bagged for disposal (pg. 31 and contact report).

- [redacted] CFU/yr (PV per strain, see calculations above)
- 2 day/yr (p. 28)
- 0.5% loss to water, air, incineration, or landfill (per Dust Model)
- Biosafety cabinet capture efficiency of 95% (per Dust Model for laboratory fume hoods)
- HEPA filter control efficiency of 99.97% (per Dust Model)
- Autoclaving sterilization efficiency 99.9999% (per GS)

Calculations:

Annual total:

= (PV) x (0.5% dust release) x (capture efficiency) x (control efficiency) x (1 – sterilization efficiency)

= [redacted]) x (0.005) x (0.95) x (0.9997) x (1-0.999999)

= [redacted] CFU/year (per strain)

Per day:

= ([redacted] CFU/year)/(2 days/yr)/(1 site)

= [redacted] CFU/site-day (per strain)

2) Other solid wastes

Amount: negligible

Basis: No other sources of release to this medium have been identified other than potential releases from residue in equipment and PPE. Submission and technical contact state that any solid wastes, including PPE, are autoclaved and double bagged for disposal (pg. 31 and contact report). Per GS, autoclaving and the small volume of waste expected results in negligible releases.

STACK AIR:

1) From: Handling solid seed coating – captured and uncontrolled particles

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)(1 site, 2 days/site-yr)

Basis: Submission does not estimate releases solid handling operations. NCD uses the total PV

and the 0.5% residual model (Dust Model) to calculate these releases. Technical contact indicates that operations occur in a biosafety cabinet. The Dust Model does not have capture efficiency information for biosafety cabinets, so NCD uses the default capture efficiency for a laboratory fume hood of 95%. Air emissions from the biosafety cabinets are ventilated through HEPA filters. NCD uses the default control efficiency for HEPA filters of 99.97% from the Dust Model.

- [redacted] (PV per strain, see calculations above)
- 2 day/yr (p. 28)
- 0.5% loss to water, air, incineration, or landfill (per Dust Model)
- Biosafety cabinet capture efficiency of 95% (per Dust Model for laboratory fume hoods)
- HEPA filter control efficiency of 99.97% (per Dust Model)

Calculations:

Annual total:

= (PV) x (0.5% dust release) x (capture efficiency) x (1-control efficiency)

= [redacted] CFU/yr) x (0.005) x (0.95) x (1-0.9997)

= [redacted] CFU/year (per strain)

Per day:

= ([redacted] CFU/year)/(2 days/yr)/(1 site)

= [redacted] CFU/site-day (per strain)

2) Aerosols

Amount: negligible

Basis: Seed coating operations are not expected to generate aerosols containing the TERA. NCD's standard assumption is to consider air releases from this activity to be negligible (per the biotech GS; consistent with past biotech cases).

OTHER WATER: Negligible

Basis: No sources of release to this medium have been identified other than potential releases from residue in laboratory equipment. NCD's standard assumption for treatment of laboratory-scale equipment (<10 liters) is that releases are negligible (per the biotech GS; consistent with past biotech cases). The technical contact indicated that all waste containing the subject organisms will be deactivated prior to disposal.

OTHER INCINERATION: Not expected

Basis: No sources of release to this media have been identified (nor are they typically expected, per NCD generic scenario).

**OCCUPATIONAL EXPOSURE**

Submission does not provide worker exposure estimates for seed coating formulation and application. These operations occur in a laboratory setting with the use of biosafety cabinets (see contact report). NCD assumes that some inhalation and dermal exposure in the laboratory setting as a worst case for potential sampling and transfer activities within the biosafety cabinets. Because the subject organisms may be in either solid or liquid form, NCD assesses inhalation and dermal exposures to both forms.

Number of Total Workers: 3

Basis: The submission estimates that 2 to 3 employees will be involved in applying the subject organisms to the seed and shipping the material (NCD assumes 3 employees) (p. 28).

Days/yr: 2

Basis: The submission estimates that seed coating will occur over approximately 2 days (p. 28).

PPE: The submission indicates that workers wear PPE (p. 28). The technical contact clarifies that PPE includes lab coats, gloves, goggles and that workers work within biosafety cabinets (see contact report).

**Summary of the Occupational Exposure Estimates:**

| Worker Activity | Exposure |
|---|---|
| **Inhalation** | |
| Laboratory activities | 7-22 CFU/day (2 days/site-yr)<br>Up to 3 workers |
| Handling solid coating | $3.6 \times 10^6$ to $1.2 \times 10^7$ CFU/day (2 days/site-yr)<br>Up to 3 workers |
| **Dermal** | |
| Handling liquids – bench scale | [redacted] CFU/day (2 days/site-yr)<br>Up to 3 workers |
| Handling solids | [redacted] CFU/day (2 days/site-yr)<br>Up to 3 workers |

INHALATION:

1) From: Laboratory activities

Amount of Exposure:
3 workers, 2 days/year
7-22 CFU/day

Basis:

Although air releases from the laboratory are expected to be negligible and inhalation exposures may be mitigated by use of standard aseptic techniques, NCD's biotech generic scenario recommends assuming some inhalation exposures in a laboratory setting as a worst case. The recommended method for estimating potential inhalation exposures is to take the most applicable area monitoring data collected by NIOSH in a fermentation facility and multiply it by an estimate of the exposure duration. The NIOSH study listed the CFU concentration in a laboratory setting to be in the range from 32 to 103 CFU/$m^3$.

Consistent with other recent GEM evaluations, the assumptions (and corresponding data) for exposure from 10 minutes of pipetting were used as analogous data to represent potential exposures to the microorganisms of this TERA during laboratory operations.

- $[CFU]_{WA}$ = 32 - 103 CFU/$m^3$ (GS estimate for laboratory setting)
- I (inhalation rate)= 1.25 $m^3$/hr
- H = hours per day = 10 min = 0.167 hrs (NCD assumption). This is area monitoring data in contrast to personal breathing zone monitoring. In using area monitoring data, an assumption needs to be made about the duration the worker is proximal to the location of the bioaerosol. NCD typically assumes a short duration of time for the worker to be exposed to bioaerosols during this part of the process.
- 2 days/yr (p. 28)
- 3 workers/site (p. 28)

Calculation:

$E_I = (I)x(h)x([CFU]_{WA})$ (per GS)

= (1.25 $m^3$/hr)x(0.167 hr/day)x(32 - 103 CFU/$m^3$)

= 7-22 CFU/day

2) From: Handling solid coating

Amount of Exposure:

3 workers, 2 days/yr
3.6 X $10^6$ to 1.2 X $10^7$ CFU/day

Basis:

NCD assumes that potential inhalation exposure may occur during handling of the solid coating. NCD uses the EPA Small Volume Handling Model to estimate exposures.

- 0.0477 to 0.161 mg exposure/kg solid handled (EPA Small Volume Handling Model, less than 54 kg of solid containing the PMN handled per site-day)
  - [redacted] CFU/yr (PV per strain, see calculations above)
  - [redacted] CFU/g (p. 28)
  - 1,500 g produced per day [calc: [redacted] (2 days/yr) / [redacted] CFU/g) = 1,500 grams/day = 1.5 kg/day]
- 2 day/yr (p. 28)
- 3 workers (p. 28)

Calculation:

= (0.0477 to 0.161 mg exposure/kg solid handled) x (1,500 g handled/day) x (kg/1000g) x [redacted] CFU/g) x (g/1000 mg)

= 3.6 X $10^6$ to 1.2 X $10^7$ CFU/day

DERMAL:

1) From: Bench scale handling of liquids

Amount of Exposure:
3 workers, 2 days/yr
[redacted] CFU/day

Basis:

Biotech generic scenario. The potential dermal dose rate is the product of NCD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream. For bench scale handling of liquids, the NCD standard dermal factor is <1.1 ml/day. This factor can be used with the concentration of the TERA:

- [redacted] CFU/ml (p. 28)
- C = liquid transfer - 1 hand, the NCD standard dermal factor is (535 $cm^2$/day)(0.7 to 2.1 mg/$cm^2$)(1 g/1000 mg)(1 mL/g) = 0.4 to 1.1 mL/day (NCD assumes 1 hand transfer for laboratory)
- 2 days/yr (p. 28)

- 3 workers/site (p. 28)

Calculation:

$E_D = ([CFU]_P) \times C$ (per GS)

= ([redacted] CFU/ml) x (1.1 ml/day)

= [redacted] CFU/day

2) From: Dermal exposure from solid handling activities (transfers to / from coating vessel)

Amount of Exposure:
3 workers, 2 days/yr
[redacted] CFU/day

Basis:
Per the biotech GS, the potential dermal dose rate is the product of NCD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream.

- [redacted] CFU/ml in solid (p. 28)
- C = solid (NCD assumes 2 hand transfer for transfers), the NCD standard dermal factor is (3,100 mg/day)(1 g/1000 mg)(1 mL/g) = up to 3.1 mL/day
- 2 days/yr (p. 28)
- 3 workers/site (p. 28)

Calculation:
$E_D = ([CFU]_P) \times C$ (per GS)

= ([redacted] CFU/mL)x(3.1 mL/day)

= [redacted] CFU/day

INITIAL REVIEW ENGINEERING REPORT [redacted]

TERA: R21-0001

USE: Field Application (Seed Planting)

Sites/Locations: [redacted] (p. 29)

[redacted]

Days/site-yr: 2 days/site

Basis: Technical contact estimates 1 – 2 days/year per site for the planting of seeds at field test sites (see contact report).

**PROCESS DESCRIPTION**

Seeds coated in the strain or the seed coating formulation will be provided to each field test site (see contact report). At the field test sites, either of the following will occur (see contact report):

1. Coated seeds will be planted with automated machinery.
   OR
2. Seed coating formulation will be injected into the ground at the time uncoated seeds are planted. The formulation will be injected with automated machinery (referred to as

in-furrow application). If in-furrow application occurs, the seed coating formulation will be liquid.

The test material will have a concentration of about [redacted] CFU/mL in liquid and [redacted] CFU/mL in solid (p. 28). The total expected amount applied per test site is about [redacted] CFU per strain (total volume of [redacted] CFU/yr divided by [redacted] p. 28).

**ENVIRONMENTAL RELEASE SUMMARY**

After testing is complete, at harvest, seeds should be distracted and buried under 2 ft of soil (p. 30). Remaining vegetative materials should be cultivated. Unused test material will be sent back to [redacted] for disposal (p. 31).

Any material used for sampling will be autoclaved before disposal and any reusable tools for sampling will be cleaned with 70% isopropyl alcohol and the solvent sent either released to directly to the field (for small amounts) or sent for incineration (for larger amounts) (p. 30 and contact report). The same cleaning and release procedures are used for the in-furrow application nozzles, if used (see contact report).

NCD assesses 100% release scenario: 99% release from seed planting (land application) and 1% release from equipment cleaning.

The following table summarizes the total releases estimated for this operation.

**Summary of the Total Release Estimates to Environment:**

| Release | Media | Amount Released (per strain) |
|---|---|---|
| Seed planting or in-furrow application | Land | [redacted] CFU/yr ([redacted] sites, 2 days/site-yr) or [redacted] CFU/site-day |
| Equipment Cleaning | Land or Incineration | [redacted] CFU/yr ([redacted] sites, 2 days/site-yr) or [redacted] CFU/site-day |
| **Total** | **All** | [redacted] **CFU/yr** |

WATER:

Amount: Not expected

Basis: Subject organisms are expected to be 100% land applied; releases to water are not expected.

AIR:

Amount: Not expected

Basis: Subject organisms are expected to be within the seed coating or in a formulation that is injected directly into the ground with automated machinery; releases to air are not expected.

LAND:

Per submission and technical contact, subject organisms will be either coated on seeds received by the field test sites or in a liquid coating formulation that is applied in-furrow at the test sites through injection into the soil. After conclusion of the trial and associated sampling, seeds will be distracted and buried under 2 ft of soil. Remaining vegetative materials will be field cultivated (p.30).

[redacted] (p. 30). These setbacks will significantly reduce the likelihood of the movement of the test organisms outside of the experimental area, even under extreme conditions.

1) From: Seed Planting or In-Furrow Injection

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)([redacted] sites, 2 days/site-yr)

Basis: The total expected amount applied per test site is about [redacted] CFU per strain (total volume of [redacted] CFU/yr divided by [redacted] sites, p. 28). Technical contact estimates plating will occur over 1 to 2 days/site-yr (see contact report). NCD assumes 99% release from seed

planting / in-furrow application (assuming 1% equipment residual).

Calculations (based on submitter information):

Annual total:

= ([redacted] CFU/site-year) x ([redacted] sites) x (1-0.01 equipment residual)

= [redacted] CFU/yr (per strain)

Per site-day:

= [redacted] CFU/yr) / [redacted] sites) / (2 days/yr)

= [redacted] CFU/site-day (per strain)

LAND or INCINERATION:

Per submission, any reusable tools for sampling will be cleaned with 70% isopropyl alcohol and the solvent sent either released to directly to the field (for small amounts) or sent for incineration (for larger amounts) (p. 30 and contact report). The same cleaning and release procedures are used for the in-furrow application nozzles, if used (see contact report).

1) From: Equipment Cleaning

Amount:

[redacted] CFU/yr (per strain)
[redacted] CFU/site-day (per strain)([redacted], 2 days/site-yr)

Basis: The total expected amount applied per test site is about [redacted] CFU per strain (total volume of [redacted] CFU/yr divided by [redacted], p. 28). Technical contact estimates plating will occur over 1 to 2 days/site-yr. NCD assumes 1% equipment residual.

Calculations (based on submitter information):

Annual total:

= ([redacted] CFU/site-yr) x [redacted]) x (0.01 equipment residual)

= [redacted] CFU/yr (per strain)

Per site-day:

= [redacted] CFU/yr) / ([redacted] / (2 days/yr)

= [redacted] CFU/site-day (per strain)

**OCCUPATIONAL EXPOSURE**

Submission does not provide worker exposure estimates for seed planting. Subject organisms are present in liquid formulation or within a solid coating affixed to seeds. NCD assesses exposures to liquid subject organisms. Because the subject organisms are entrained in the coating, EPA assumes that inhalation exposures will not occur from solid coating and dermal exposures are non-quantifiable (consistent with past case [redacted]).

Number of Total Workers: 2 per site (8 total over all sites)

Basis: Technical contact estimated 1-2 workers (see contact report).

Days/yr: 2

Basis: Technical contact estimated 1-2 days/year (see contact report).

PPE: The submission indicates that workers are instructed to wear appropriate PPE (p. 30).

**Summary of the Occupational Exposure Estimates:**

| Worker Activity | Exposure |
|---|---|
| **Inhalation** | |
| All | Not expected |
| **Dermal** | |
| Unloading and transferring liquid formulation into in-furrow application system | [redacted]/day (2 days/site-yr)<br>Up to 8 workers |
| Unloading of seeds coated in TERA | Non-quantifiable |

INHALATION: Not expected

Basis: Subject organisms are coated onto seeds prior to delivery or are within liquid formulation that is injected into the ground with automated machinery; inhalation exposures are not expected from solid coating (subject organisms are entrained in the coating) or worker handling of liquid formulation (application is an injection process and does not involve spraying).

DERMAL:

1) From: Unloading and transferring liquid formulation into in-furrow application system

Amount of Exposure:

8 workers (2 workers/site)
[redacted] CFU/day, up to 2 days/site-yr

Basis:

Per the biotech GS, the potential dermal dose rate is the product of NCD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream.

- $[CFU]_B$ = [redacted] CFU/mL (p. 28)
- C = liquid, the NCD standard dermal factor is (1,070 $cm^2$/day)(up to 2.1 mg/$cm^2$)(1 g/1000 mg)(1 mL/g) = up to 2.2 mL/day (NCD assumes 2 hand transfer for unloading)

Calculation:

[redacted] CFU/mL)x(2.2 mL/day)

= [redacted] CFU/day

2) From: Unloading of seeds coated in TERA

Amount of Exposure: Non-quantifiable

Basis: Field test sites may receive seeds coated with the subject organisms. Because the subject organisms are entrained in the coating, dermal exposures are considered to be non-quantifiable (per past cases).

[redacted] **CONTACT REPORT for TERA R21-0001**

Submitter: [redacted]

DATE: January 4, 2021 and January 5, 2021 calls
Additional information provided via email
(Summarized at end of contact report)

Person Contacted: [redacted]

Telephone: [redacted]

[redacted]

Laboratory propagation

- Q: Where does the production of the TERA occur? At the [redacted]
- A: The material produced at the [redacted] site and is then sent to the [redacted] site where it will be coated onto seeds or formulated for use at the field test sites.

- Q: The submission states the production occurs for approximately 3 to 4 days – is this the total annual number of days?
- A: Yes, total annual.

- Q: Is this a lab-scale operation?
- A: Yes

- Q: What is the method of disposal of wastes?
- A: All wastes are deactivated prior to disposal. Solid wastes are autoclaved. Liquid waste treated with [redacted]. There is an effluent capture system that routes to the wastewater evaporation ponds at the [redacted] site.

- Q: Do you have information on the inactivation practices at your facilities? Is it 6-log reduction or greater?
- A: [redacted] facility. They deactivate everything before wastewater is deactivated. Essentially 100% treatment. Would need to get the information.

- Q: What type of PPE?
- A: Lab coats, gloves, goggles. Worked in biosafety

cabinets at both facilities.

Formulation and Seed Coating:

- Q: Where does seed coating occur?
- A: Seed coating is the preferred approach and would occur at the [redacted] site. However, the submitter is still working on the formulation and it may end up being applied in-furrow at the field test sites (injected into the soil with automated machinery at the time the seeds are planted).

- Q: What is the process used to coat the seeds?
- A: Seed coating is done in a piece of equipment called a hege bowl. The hope is to have the seed coating formulation be a dry powder, but it could also be a liquid formulation.
- The[redacted] facility would receive the coating from the [redacted] facility in either liquid or solid form.
- See updated information provided by email (summarized below).

- Q: The submission states seed coating occurs for approximately 2 days – is this the total per year?
- A: Yes, that is the annual estimate.

- Q: Is the seed coating operation a lab-scale operation?
- A: Yes

- Q: What wastes do you expect from this process? What is the method of disposal of wastes?
- A: The submitter does not expect any waste from the coating process. Potentially from equipment cleaning.
- All wastes are deactivated prior to disposal. Solid wastes are autoclaved. Liquid waste treated with bleach. There is an effluent capture system that routes to the wastewater evaporation ponds at the California site.

- Q: Do you have information on the inactivation practices at your facilities? Is it 6-log reduction or greater?
- A:[redacted] facility. They deactivate everything before wastewater is deactivated. Essentially 100% treatment.

- Q: At the [redacted] site, what happens to water from the ponds? Are they evaporation ponds?

- A: The water seeps into the soil / evaporates. Does not discharge.

- Q: Do you have air emission controls at the [redacted] site? Such as for potential air emissions from handling of the TERA in solid coating formulation? Are the biosafety cabinets vented to filter?
- A: Yes, they vent through filters – this is a permitting requirement.

- Q: Will the seed coating be entrained on the seed or is it loose?
- A: It's bound / affixed to the seed.

- Q: What type of PPE?
- A: Lab coats, gloves, goggles. Worked in biosafety cabinets at both facilities.

Use (Seed Planting):

- Q: Is planting performed by the field test sites or [redacted] employees?
- A: Field test sites.

- Q: Over how many days do you expect planting to occur at each site?
- A: Guess 1 – 2 days for planting.

- Q: How many workers will be involved in the planting of the seeds at the test sites?
- A: Guess 1 – 2 workers since its just planting. The sites use automated equipment for planting seeds and injecting the TERA formulation into the soil (if seed coating does not occur). Therefore, the TERA formulation is not really handled by the person.

- Q: The submission states that any reusable sampling equipment is cleaned with IPA. Is that used solvent expected to be incinerated?
- A: Rinsing occurs between sampling. The amount of IPA needed to rinse shovels and trowels would be small and would just be done in the field (land release).

- Q: Do you expect any wastes from the seed planting process?

- A: Doesn't expect anything unless in-furrow application. If in furrow, the injection nozzles would be rinsed with solvent and the waste solvent would either be released to the field (if a small amount) or incinerated (if a large amount).

Overall:

- Q: Do the subject organisms form spores?
- A: Yes, capable of forming spores. Testing showed that there is no significant presence after four weeks, so they don't expect the subject organisms to persist for very long.

Additional information provided in February 11, 2021 email:

- Q: What is the volume of strain manufactured?
- A: Approximately [redacted] of formulated material concentrated to [redacted]CFU/g.
- Reflected in the amended submission.

- Q: Describe the laboratory propagation stage.
- Updated process description provided in the amended submission.

- Q: Can you provide any details/a sketch of the hege bowl coating operation of the subject organism onto the seeds?
- A: We will be using closable plastic food grade containers for performing seed treatment in a biosafety cabinet.